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Patents Form 1/77

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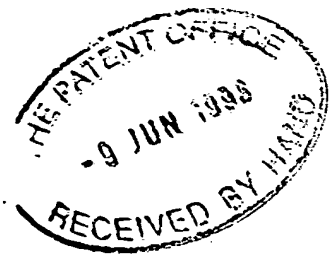

1/77

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1. Your reference

NCB/P21046GB

2. Patent application number *(The Patent Office will fill in this part)*

9 JUN 1998

9812432.4

9 JUN 1998

3. Full name, address and postcode of the or of each patent applicant *(underline all surnames)*

QUEEN MARY & WESTFIELD COLLEGE

Mile End Road
London E1 4NS .

(ADDITIONAL APPLICANT ON SEPARATE SHEET)

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

(GB)

4. Title of the invention

PREDICTIVE TEST

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

KILBURN & STRODE
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LONDON
WC1R 4PJ

Patents ADP number *(if you know it)*

125001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application number	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

YES

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body. See note (d))

Patents Form 1/77

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Priority documents	:	-
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	:	-
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Request for substantive examination *(Patents form 10/77)*	:	-
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11. I/We request the grant of a patent on the basis of this application.

Signature: Kilburn & Strode

Date: 9 June 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

Nick C. Bassil
Tel: 0171-539 4200

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ADDITIONAL APPLICANT

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PREDICTIVE TEST

The present invention relates to a test which can be used to predict pre-eclampsia in pregnant women.

Pre-eclampsia is a disorder of human pregnancy which affects around 5 to 10% of pregnancies. The underlying cause of pre-eclampsia remains unclear in spite of extensive clinical and basic research. Pre-eclampsia is the definition given to the condition in pregnancy in which elevated blood pressure is associated with proteinuria. Pre-eclampsia is distinct from eclampsia which is additionally associated with convulsions. Pre-eclampsia is defined in Souhami & Moxham *Textbook of Medicine*, Second edition, Churchill Livingstone (1994), as an abnormal rise in blood pressure between the first and second halves of pregnancy of ≥ 30/20 mmHg, with abnormal urate levels of >0.35 mmol/l at 32 weeks or >0.4mmol/l thereafter, associated with proteinuria, impaired renal function and clotting disorders. The consequences of pre-eclampsia are serious and include reduced uteroplacental perfusion, foetal growth retardation, pre-term birth, and increased foetal and maternal morbidity and mortality.

There have been many attempts to provide a reliable predictive test have suggested assays for the levels of circulating biochemical markers in the mother's blood but to date the scientific literature on this issue is contradictory and inconclusive. The following hormones have all been identified as possible markers in an elevation of levels might be predictive of pre-eclampsia in maternal plasma: progesterone, oestradiol, human chorionic gonadotrophin (hCG), corticotrophin-releasing factor (CRF), adrenocorticotrophin. Conversely, levels of oestriol, human placental lactogen and cortisol are unchanged or decreased. Whilst circulating CRF has been proposed as a prognostic marker for pre-eclampsia, treatment of hypertension does not influence maternal CRF levels and nor has any correlation been found between CRF levels and mean blood pressure.

Other possible markers which have been suggested are Activin A and Inhibin A. Activin is a hypophysiotrophic factor produced by the placenta which is know to act as a growth factor having activity in modulating cell growth and differentiation. Currently, there are three forms of activin which are recognised to exist as homodimeric proteins: Activin A ($\beta_A\beta_A$), Activin AB ($\beta_A\beta_B$) and Activin B ($\beta_B\beta_B$) in which the subunits are linked by disulphide bridges. Inhibins are heterodimeric proteins consisting of $\alpha\beta_A$ (Inhibin A) and $\alpha\beta_B$ (Inhibin B) subunits also linked by disulphide bridges. Additionally monomeric inhibin α subunits are present in the circulation and follicular fluid. Inhibin is thought to have an endocrine role which inhibits pituitary production of follicle-stimulating hormone (FSH). Muttikrishna *et al* (*The Lancet* **349** 1285-1288 (1997)) have proposed that Activin A and Inhibin A might be suitable markers for the onset of pre-eclampsia. These proteins were suggested because they were thought to be more sensitive markers than hCG or corticotrophin-releasing hormone where there is a considerable overlap in elevated hormone levels between control and pre-eclamptic women.

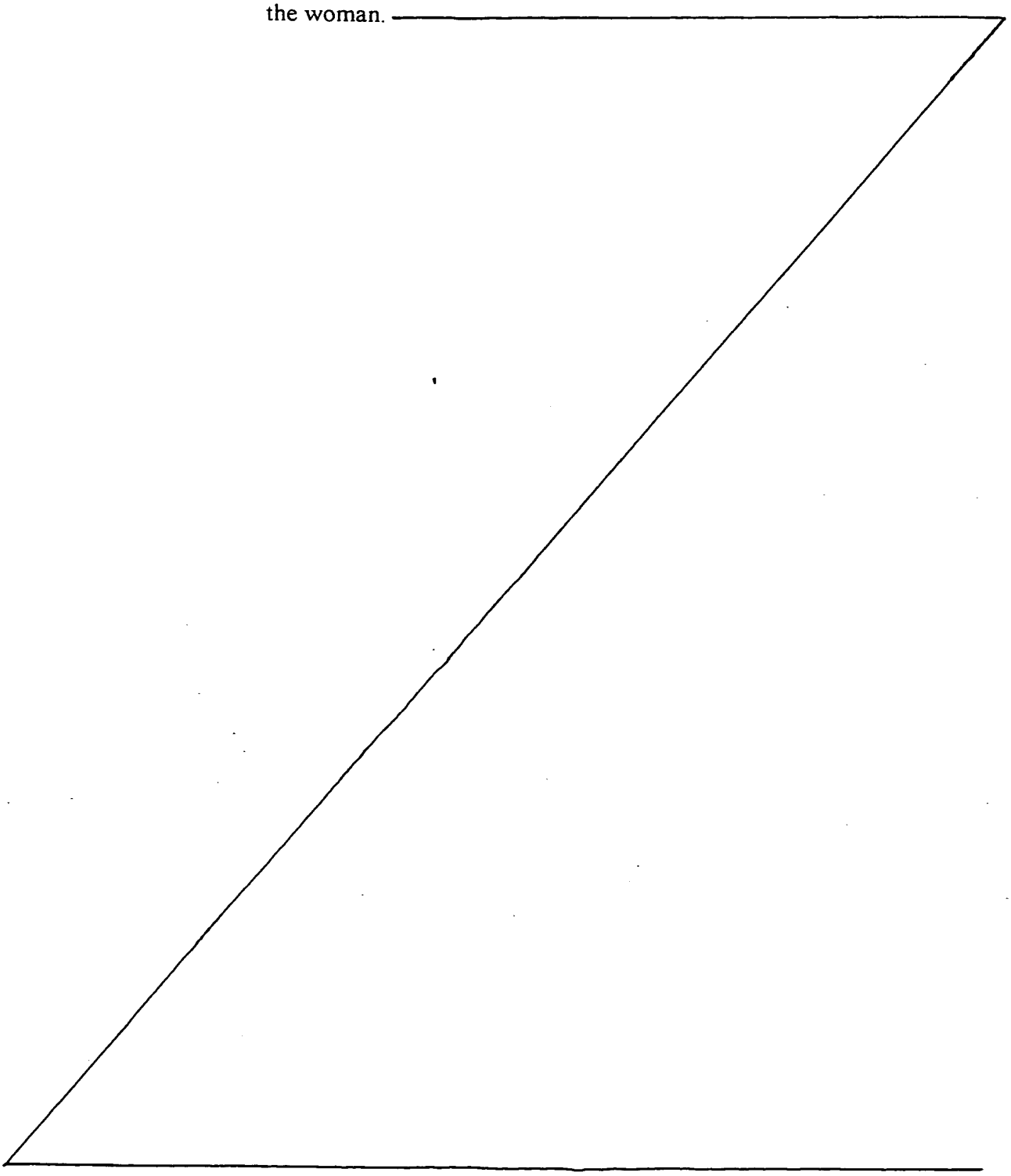
However, it has now been found that a predictive test for pre-eclampsia which is based on levels of human chorionic gonadotrophin (hCG) and Inhibin A can in fact provide a surprisingly improved level of predictiveness over previously known tests.

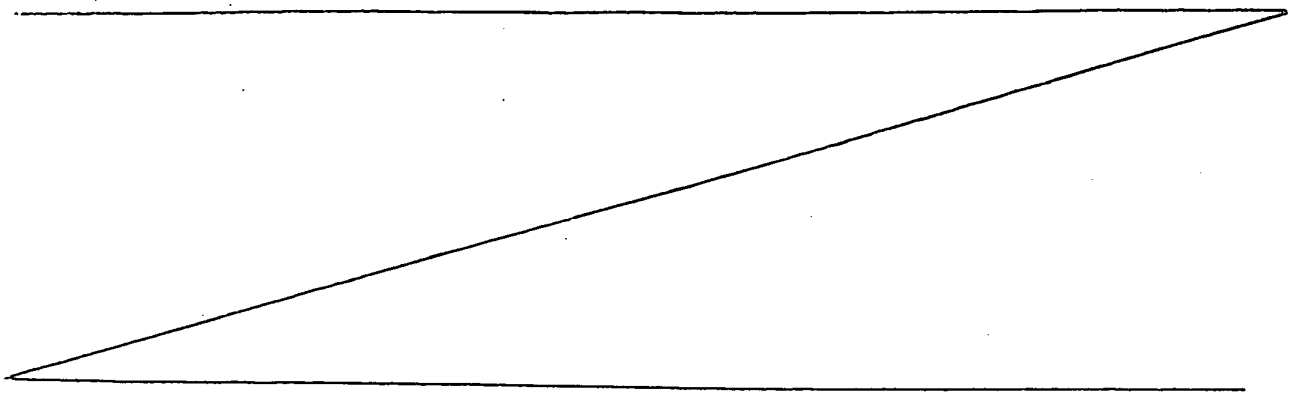
According to a first aspect of the invention there is provided a method of predicting the onset of pre-eclampsia in a pregnant woman, the method comprising the steps of:

(a) obtaining a sample of blood from the woman;

(b) subsequently assaying the sample for the levels of human chorionic gonadotrophin (hCG) and Inhibin A present in the sample; and

(c) comparing the levels of human chorionic gonadotrophin (hCG) and Inhibin A present in the sample with those in a control sample to provide a prediction of the probability of the onset of pre-eclampsia in the woman.





Subjects, methods, and results

We used serum collected between 1973 and 1975 from the John Radcliffe Maternity Hospital, Oxford. Pre-eclampsia was defined as (i) a rise in systolic and diastolic pressure during pregnancy of 30 and 20 mm of mercury respectively, compared with the level found at the first antenatal booking visit; (ii) proteinuria greater than 10mg % in a mid-stream urine sample; (iii) renal impairment as judged by the elevation of plasma uric acid levels of 6 mg % or more. Nineteen women had blood samples taken after 12 weeks' gestation stored at -40°C. Nine women had one sample, seven had two samples, and three had three samples. For each sample we identified three controls, selected at random from the patients attending the hospital who had provided a blood sample at the same gestational age in the same calendar quarter and were the same age. Neither cases nor controls were associated with Down's syndrome or neural tube defects. Serum alphafetoprotein (AFP) and free β-human chorionic gonadotrophin (hCG) were measured using the Wallac-Delfia kit, unconjugated oestriol (uE_3) using the Ortho Clinical Diagnostics kit, and inhibin A using the assay kit produced by Serotec. One sample was sufficient only to measure inhibin A. For each serum marker, the logs of the medians for the controols were plotted by gestational age and a regression line fitted. The predicted marker values for each gestational age were estimated. All markers were expressed as multiples of their predicted median values for the controls

(that is, MoMs).

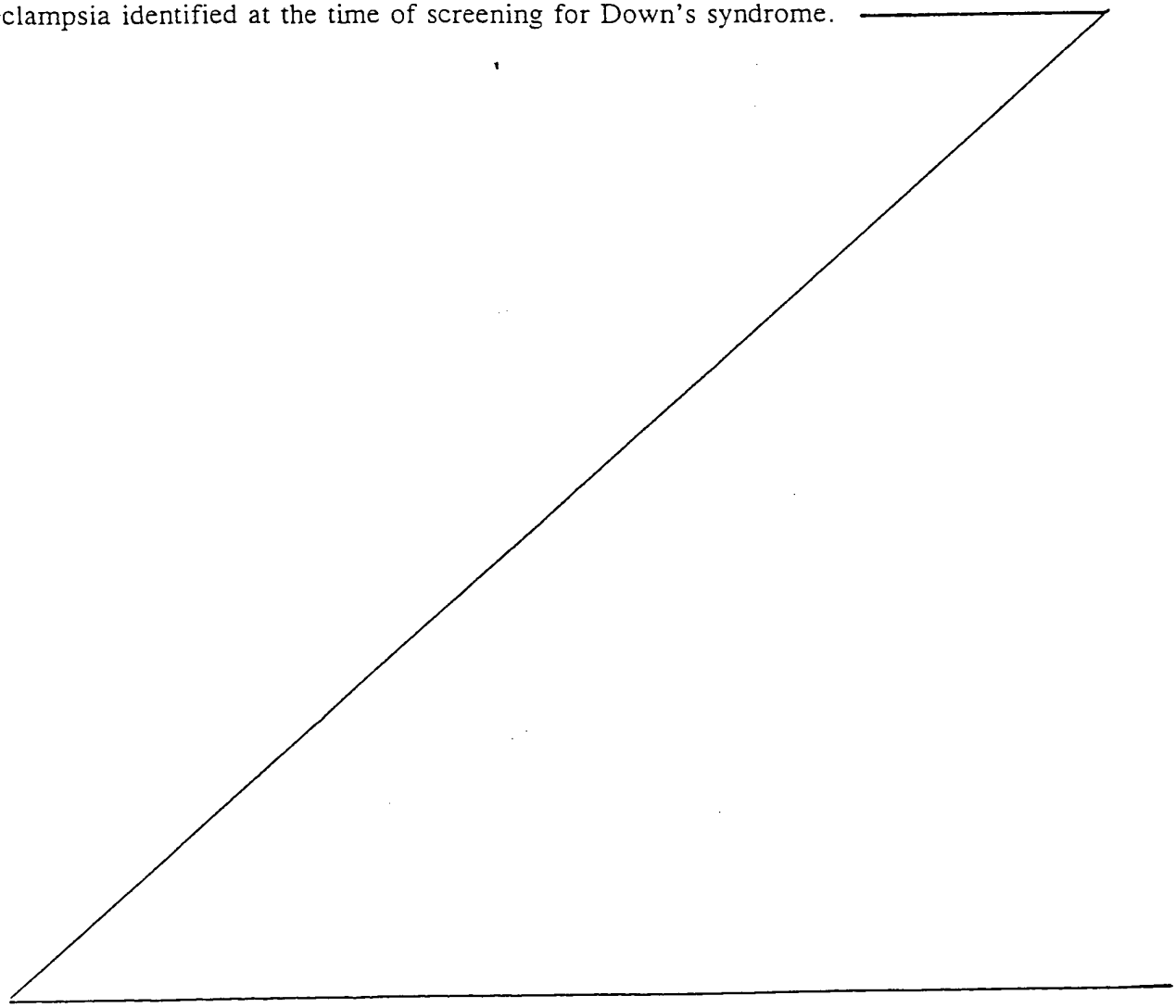
All analyses were also completed by using the marker values for each case expressed as a multiple of the median value of its three controls. This removes the need to model the relationship of the markers with gestational age. The results did not differ significantly from those presented here.

The data were analysed using robust regression with the cluster option in STATA[6] to take account of repeat samples of some of the women. Table 1 shows the results for the four markers used classified according to the onset of proteinuria. Inhibin A and free β-hCG values are raised in the pregnancies with pre-eclampsia and the level increases with decreasing time prior to proteinuria and is highest in women after the diagnosis of the disorder. Within three weeks of the onset of proteinuria the mean inhibin A value was 3.18 times the median for controls (95% CI, 1.98-5.11), and the mean free β-hCG 3.43 (1.58-7.42). Even 10 weeks prior to the onset of proteinuria these two markers were elevated. The mean uE_3 was significantly reduced in the controls, within three weeks of the onset of proteinuria, MoM = 0.51 (95% CI, 0.42-0.62), but appears to rise again after onset of proteinuria.

Comment

Our results show that inhibin A and free β-hCG are useful for second trimester serum markers for pre-eclampsia. Each provided some independent predictive information because they were not totally correlated. Figures 1 and 2 and table 2 demonstrate that both the inhibin A and free β-hCG data fit log Gaussian distributions reasonably well. Table 2 shows the

observed and expected (using the log Gaussian model) number of affected pregnancies above specified inhibin A and free β-hCG levels. The correspondence is good. Based on the multivariate Gaussian model using the parameters in table 3 (based on results prior to the onset of proteinuria) in combination they yield a 40% detection rate for a 5% false-positive rate. The reduction in uE_3 needs to be investigated in further studies. These estimates are tentative because they are based on small numbers but provide an indication of the potential use of Down's syndrome screening markers in the prediction of pre-eclampsia. It provides the opportunity to undertake randomised prevention trials in women at high risk of pre-eclampsia identified at the time of screening for Down's syndrome.



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1. Muller F, Savey L, Le Fiblec B, Bussieres L, Ndayizamba G, Colau JC, Giraudet P. Maternal serum human chorionic gonadotropin level at fifteen weeks is a predictor for preeclampsia. *Am J Obstet Gynecol* 1996;175:37-40.

2. Ashour A, Lieberman ES, Wilkins Haug LE, Repke JT. The value of elevated second-trimester beta-human chorionic gonadotropin in predicting development of preeclampsia. *Am J Obstet Gynecol* 1997;176:438-44.

3. Hsu CD, Chan DW, Iriye B, Johnson TRB, Hong SF, Repke JT. Elevated serum human chorionic gonadotropin as evidence of secretary response in severe preeclampsia. *Am J Obstet Gynecol* 1994;170:1135-8.

4. Wenstrom KD, Owen J, Boots LR, DuBard MB. Elevated second-trimester human chorionic gonadotropin levels in association with poor pregnancy outcome. *Am J Obstet Gynecol* 1994;171:1038-41.

5. Muttukrishna S, Knight PG, Groome NP, Redman CWG, Ledger WL. Activin A and inhibin A as possible endocrine markers for pre-eclampsia. *Lancet* 1997;349:1285-8.

6. Stata Corporation. 1997. Stata Statistical Software: Release 5.0. College Station, TX: Stata Corporation.

Legends for figures:

Figure 1: Probability plot of the inhibin levels in maternal serum in pre-eclampsia pregnancies (n=23) and unaffected pregnancies (n=96) collected before the onset of proteinuria. MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

Figure 2: Probability plot of the β-hCG levels in maternal serum in pre-eclampsia pregnancies (n=22) and unaffected pregnancies (n=93). MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

Table 1 Specified serum marker levels in pregnancies with preeclampsia according to timing of collection of serum sample relative to onset of proteinuri

Collection of serum sample relative to onset of proteinuria	Median gestation of onset of proteinuria	Number of women	Number of samples	Median gestation of serum sample	Geometric Mean (MoM) values (95% confidence interval)			
					Inhibin A	AFP	Free β-hCG	uE_3
Over 11 weeks before	29.9	10	10	12.1	1.00 (0.75-1.32)	0.82 (0.61-1.11)	1.29 (0.95-1.76)	0.96 (0.48-1.94)
10-4 weeks before	29.4	6	6	21.5	1.26 (0.66-2.41)	1.13 (0.78-1.64)	2.09 (1.24-3.54)	0.87 (0.67-1.14)
3-0 weeks before*	28.9	6	7†	27.9	3.18 (1.98-5.11)	1.60 (0.58-4.42)	3.43 (1.58-7.42)	0.51 (0.42-0.62)
Up to 3 weeks after proteinuria*	29.9	5	9	32.3	6.66 (3.80-11.68)	1.36 (0.63-2.95)	3.98 (2.52-6.31)	0.93 (0.66-1.29)
Total* (95% CI)	29.9	19	32†	23.4	2.27 (1.52-3.38)	1.14 (0.83-1.57)	2.34 (1.66-3.28)	0.82 (0.60-1.12)
Total prior to onset proteinuria*	29.8	16	23†	21.1	1.49 (1.03-2.16)	1.07 (0.76-1.52)	1.92 (1.28-2.89)	0.78 (0.54-1.13)

MoM multiples of the median

* Standard errors adjusted for more than one sample from some women

† One sample only had measurements of inhibin

Table 2 Number (and percentage) of pregnancies with preeclampsia collected before onset of proteinuria and unaffected pregnancies acording to inhibin A and free β-hCG

	Inhibin A				β-hCG			
MoM	Affected Number (%) (n=23)	Modelled* %	Unaffected Number (%) (n=96)	Modelled* %	Affected Number (%) (n=22)	Modelled* %	Unaffected Number (%) (n=93)	Modelled* %
≥0.5	21 (91%)	93%	87 (91%)	90%	22 (100%)	97%	79 (85%)	84%
≥1.0	17 (74%)	70%	42 (44%)	50%	18 (82%)	82%	46 (49%)	50%
≥1.5	11 (48%)	50%	18 (19%)	22%	13 (59%)	63%	29 (31%)	28%
≥2.0	6 (26%)	35%	13 (14%)	9%	9 (41%)	48%	17 (18%)	16%
≥2.5	6 (26%)	25%	5 (5%)	4%	7 (32%)	36%	7 (8%)	9%
≥3.0	4 (17%)	18%	3 (3%)	2%	7 (32%)	27%	5 (5%)	5%

* These percentages are estimated assuming both inhibin A and free β-hCG have log normal distributions

Table 3 Distribution parameters of inhibin A and free β-hCG in pregnancies with and without preeclampsia based on samples collected before onset of proteinuria (23 cases and 96 control samples)

	$\log_{10}$ MoM	
	Inhibin A	free β-hCG
Means		
Unaffected	0	0
Affected	.164	.284
Standard deviations		
Unafffected	.234	.297
Affected prior to onset of proteinuria	.332	.317
Correlation		
Unaffected	.198	
Affected	.899	

Figure 1

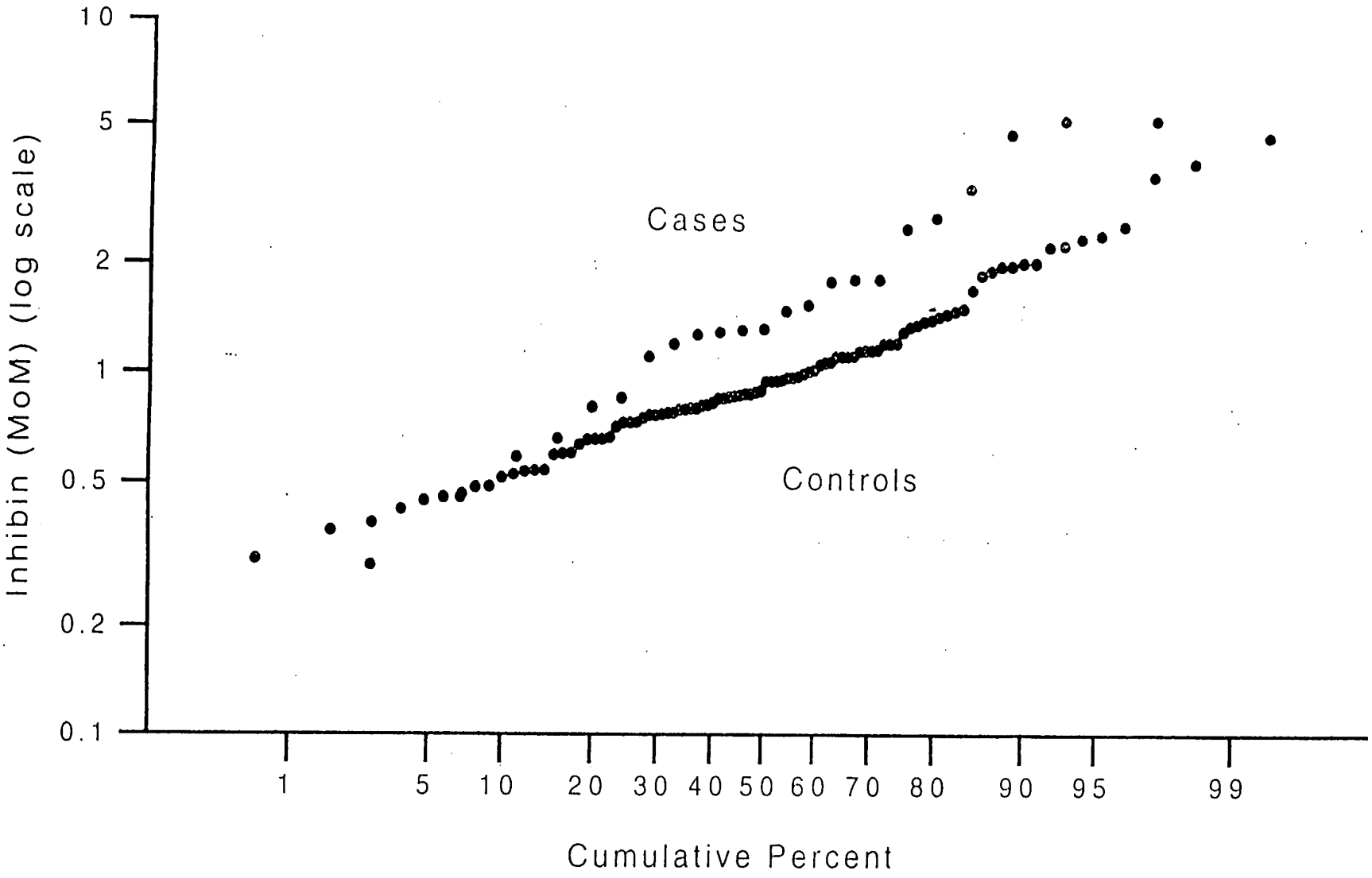
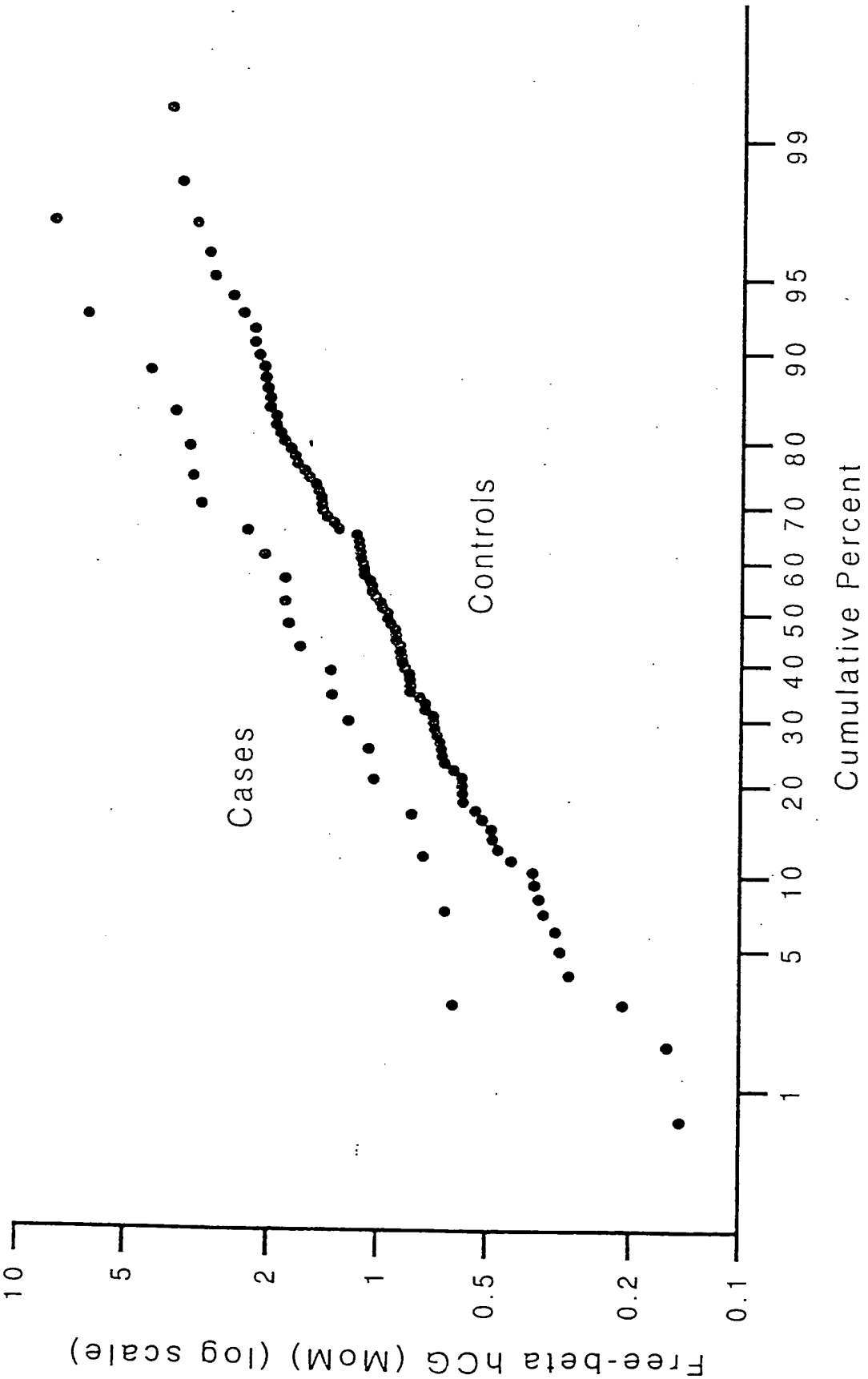

10
5
2
1
0.5
0.2
0.1
Inhibin (MoM) (log scale)
Cases
Controls
1
5
10
20
30
40
50
60
70
80
90
95
99
Cumulative Percent

Figure 2


Free-beta hCG (MoM) (log scale)
10
5
2
1
0.5
0.2
0.1
Cases
Controls
1
5
10
20
30
40
50
60
70
80
90
95
99
Cumulative Percent

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